ISSN 1364–7565 Acta Phytotax. Geobot. 54 (1): 69–75 (2003)

Intra-specific Polyploidy and Possible Occurrence of Some Genetic Types for Pollen Development in *Cayratia japonica*, Vitaceae.

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Cayratia japonica showed intra-specific polyploidy of x=20, i.e., $2n$=40 (diploid) and 60 (triploid). Individuals with $2n$=40 produced fruits, and formed 20 bivalents at metaphase I in meiosis, but their pollen fertilities widely varied from about 30 to 90%. Individuals with $2n$=60 did not produce any fruits, formed a variable number of trivalents, bivalents and univalents at metaphase I, and showed low pollen fertilities of less than 30%. The possible occurrence of some genetic types for pollen development in diploids is discussed.

Key words: *Cayratia japonica*, chromosome, meiosis, ploidy, pollen development, pollen fertility, Vitaceae

Cayratia japonica (Thunb.) Gagn. is well known as a wild climbing plant in Japan. Attentive observers know that this species contains both individuals with fruits and without fruits in nature. From a general view point, individuals without fruits (hereafter non-fruit-individuals) are frequently observed at locations under strong horticultural impact, such as newly established squares after land development, hedges and green belts along roads, while individuals with fruits (hereafter fruit-individuals) grow mainly in more natural habitats, such as the boundaries of forests, bush, and house gardens in the countryside. We want to know the reason why some plants produce fruits, but others do not. On the other hand, the two morphs can be recognized by leaf shape, namely, the fruit-individuals frequently develop trifoliate leaves, in addition to standard, pentafoliate leaves, while the non-fruit-individuals scarcely develop trifoliate leaves (our unpublished observation). At some places, both fruit- and non-fruit-individuals grow closely together, and insects similarly visit both individuals. Therefore, environmental conditions do not seem to be the limiting factor in fruit formation. Some species-specific situation must affect the reproductive traits. In some cases the cytogenetic aspects affect reproductive traits, such as ploidy levels and meiotic aspects (for examples; Clausen *et al.* 1945, Cleland 1972, Sakamoto 1982, Noda & Kawano 1988). In fact, the chromosome number of this species was reported as $2n$=40 in China (Huang *et al.* 1988) and 60 in Japan (Mitsukuri & Hayashi 1953). Hence, it is plausible that the differences between the fruit- and non-fruit-individuals might be due to cytological differences. To examine the hypothesis above, we examined these two morphs cytologically. In the course of the present study, additionally, we encountered peculiar variations in the fertilities of pollen of the fruit-individuals. We attempted to clarify the cause of this phenomenon and discuss the possible genetic differentiation among fruit-individuals of *C. japonica*.

Materials and Methods

The materials examined are listed in Table 1. As *Cayratia japonica* can propagate vegetatively, samples were collected at at least 100 m intervals within the same locality. In some cases, we collected seeds, which were germinated after concentrated sulfuric acid treatment for 30 min. They were planted at the Botanical Gardens, Faculty of Science, Osaka City University. Voucher specimens are kept in Osaka Museum of Natural History (OSA) and Botanical Gardens, Faculty of Science, Osaka City University.

Both somatic and meiotic chromosomes were observed with the same methods as in a previous report (Okada 1984). Microspores were stained by the Feulgen's reaction. To estimate the pollen fertility, pollen grains were stained with lacto-phenol cotton blue solution, and darkly stained and globose grains were counted as fertile grains from a total sum of 500 to 1,000 grains

Results

In this study, we observed two different chromosome numbers, 2*n*=40 and 60, within the species distributed in Japan (Figs. 1A, 3A, B). Chromosome sizes varied in length from about 2 to 1.2 µm at the somatic metaphase. All the individuals with 2*n*=40 produced fruits and good seeds, while those with 2*n*=60 did not produced any fruits (Table 1) or exceptionally produced a few fruits (data not shown). Similarly, all the individuals with 2*n*=40 were frequently observed to develop trifoliate leaves, while all those with 2*n*=60 did not (data not shown).

In meiosis, the individuals with 2*n*=40 formed 20 bivalents at metaphase I (Fig. 1B) without any exceptions. At metaphase II and anaphase II, each nucleus contained 20 chromosomes (Fig. 1C). Tetrads were formed normally (Fig. 1D). Microspores were almost the same size. At microspore cell division, 20 chromosomes were observed (Fig. 1E), and two nuclei appeared to be formed normally. In some individuals, two kinds of pollen grains, namely, normal and degenerative, were observed (Fig. 2). The degenerative pollen grains had a broken pollen wall, and their cytoplasm was lost (Fig. 2B). Pollen fertilities varied from 31% to 97% among the plants examined (Fig. 1F, Table 1).

Contrary to the individuals with 2*n*=40, the individuals with 2*n*=60 formed trivalents, bivalents and univalents, of which the number varied among cells, for example $11_{\mathrm{III}}+12_{\mathrm{II}}+3_{\mathrm{I}}$, $10_{\mathrm{III}}+14_{\mathrm{II}}+2_{\mathrm{I}}$ (Figs. 3C, D), and $9_{\mathrm{III}}+15_{\mathrm{II}}+3_{\mathrm{I}}$. At metaphase II, the pollen mother cell contained nuclei with various chromosome numbers, for instance, in some cases, both nuclei had 30 chromosomes, but in other cases, one consisted of 33 chromosomes, while the other was 27 (Fig. 4A). There were various abnormal pollen grains, i.e., with a broken pollen wall, without cytoplasm, or smaller or larger sized grains (Fig. 4B). Pollen fertilities were less than 30% (Table 1).

Discussion

In this study it became clear that the basic chromosome number of *Cayratia japonica* is 20 judging from the meiotic aspects, that is, all of the individuals observed with 2*n*=40 formed 20 bivalents, while those with 2*n*=60 formed a variable number of trivalents, bivalents and univalents. All of the fruit-individuals had 2*n*=40, namely, diploid, while all of the non-fruit-individuals possessed 2*n*=60, i.e., triploid. Probably, diploid individuals can propagate from both seeds and cuttings of the subterranean stem. On the other hand, triploid individuals may propagate only from cuttings of the subter-

TABLE 1. Localities, somatic chromosome numbers, chromosome pairing at meiotic metaphase I, fruit formation and pollen fertilities of *Cayratia japonica*.

locality	chromosome number 2n	n	fruit form-ation	pollen fertility (%)	voucher
Naruse, Machida, Tokyo	40		+[4]		Kitamura s.n. (OSA 153352)
Shizen-kansatsu-no-mori, Yokohama, Kanagawa Pref.	40		+		Kitamura s.n. (OSA 153353)
Myodaiji-cho-4, Okazaki, Aichi Pref.	40		+	63	Tsukaya 1001
Tashiro-cho, Higashi-ku, Nagoya, Aichi Pref.	40		+	84	Tsukaya 103
Kaisho-no-mori, Aichi Pref.	40	20[1]	+	56	Tsukaya 107
Okazaki Castle, Myodaiji-cho, Okazaki, Aichi Pref.	40		+	85	Tsukaya 106
Ohsumi-ga-oka, Kyotanabe, Kyoto Pref.	40		+	32	Okada 5632
Kisaichi, Katano, Osaka Pref.	40	20_{II}[2]	+	97	Okada 5637
Bot. Gard. Kisaich, Katano, Osaka Pref.	40	20_{II}[2]	+	81	Okada 5605
Hamadera, Sakai, Osaka Pref.	40		+	42	Yamamoto s.n. (OSA 149617)
Mukogawa, Nishinomiya, Hyogo Pref.	40		+	96	Okada 5623
Yakushi-machi, Tottori, Tottori Pref.	40	20_{II}[2]	+	31	Okada 5631
Campus of Tottori Univ.-1, Koyama, Tottori, Tottori Pref.	40		+	65	Okada 5629
Campus of Tottori Univ.-2, Koyama, Tottori, Tottori Pref.	40		+	76	Okada 5606
Motobu-cho, Okinawa Isl., Okinawa Pref.	40		+	85	Okada 5638
Mt. Azuma, Sendai, Miyagi Pref.	60		-[4]		Yokoyama 010920
Campus of Univ. of Tokyo, Hongo, Tokyo	60	33+27~30+30[1]	-	16	Tsukaya 105
Myodaiji-cho-1, Okazaki, Aichi Pref.	60		-	9	Tsukaya 101
Myodaiji-cho-3, Okazaki, Aichi Pref.	60	IIIs+IIs+Is[3]	-	20	Tsukaya 104
Myodaiji-cho-2, Okazaki, Aichi Pref.	60	IIIs+IIs+Is[3]	-	25	Tsukaya 102
Bot. Gard. Kisaich, Katano, Osaka Pref.	60	IIIs+IIs+Is[3]	-	16	Okada 5636
Bot. Gard. Nagai, Osaka, Osaka Pref.	60		-	28	Okamoto s.n. (OSA 153508)

[1] metaphase II

[2] metaphase I

[3] IIIs: trivalents, IIs: bivalents, Is: univalents

[4] +: produce fruits, -: does not produce fruits

ranean stem when ornamental plants are transplanted in human horticultural activities. Exceptionally, triploid individuals produced few fruits, which might have occurred due to the rather high fertility, i.e., about 9-28%.

Although all of the diploids produced fruits, they showed a wide range of variation in pollen fertility, from 31 to 97% (Table 1). Low pollen fertilities did not arise from abnormalities in meiosis, that is, all of the individuals with $2n$=40 normally advanced to meiosis, but microspore degeneration occurred during the tetrad stage and microspore cell division. In this study, we cannot specify what genetic or physiological phenomena affect microspore degeneration.

Okada (1984) observed that an artificial diploid hybrid between *Ranunculus silerifolius* and *R. chinensis* exhibited almost normal meiosis, but had a very low pollen fertility. This contradiction was explained on the assumption that multiple genes

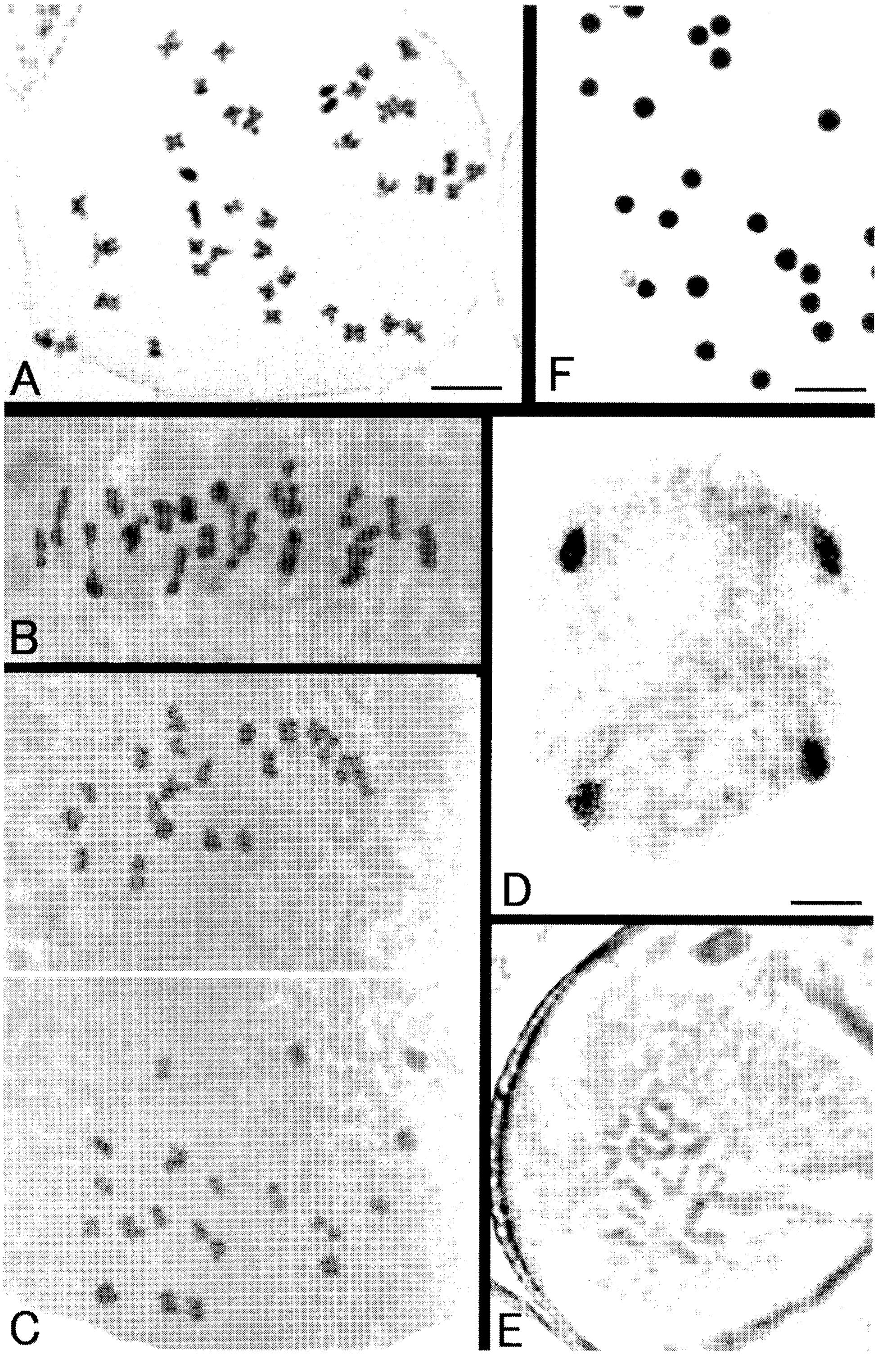

FIG. 1. Cytogenetic characteristics of the fruit-individuals of *Cayratia japonica*. A: Somatic metaphase chromosomes, $2n$=40, B: meiotic chromosomes, 20_{II} at metaphase I, C: 20 chromosomes in each nucleus at metaphase II, D: normal tetrad, E: 20 chromosomes in a normal microspore, F: pollen of an individual with $2n$=40. Bar in A, 4 μm for A, B, C and E; bar in D, 8 μm; bar in F, 100 μm.

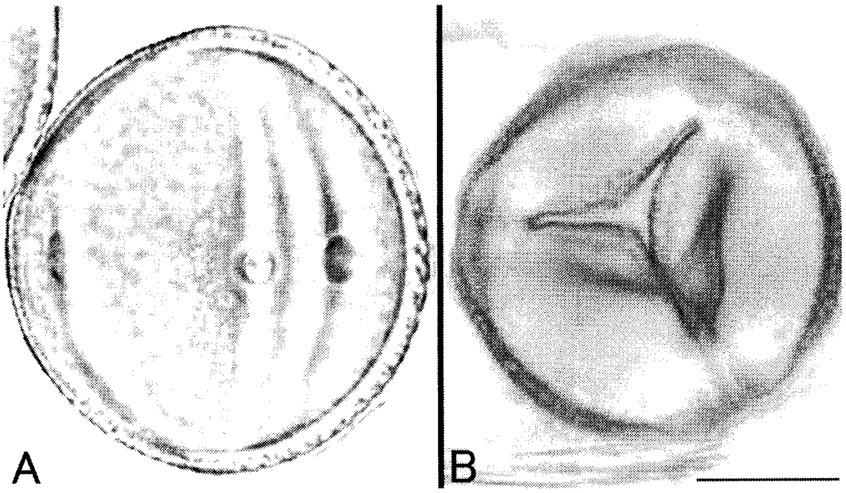

FIG. 2. Normal (A) and abnormal (B) pollen grain from individuals with $2n$=40. Note that the abnormal pollen grain is almost the same size as the normal one, but has a broken wall. Bar in B, 10 μm.

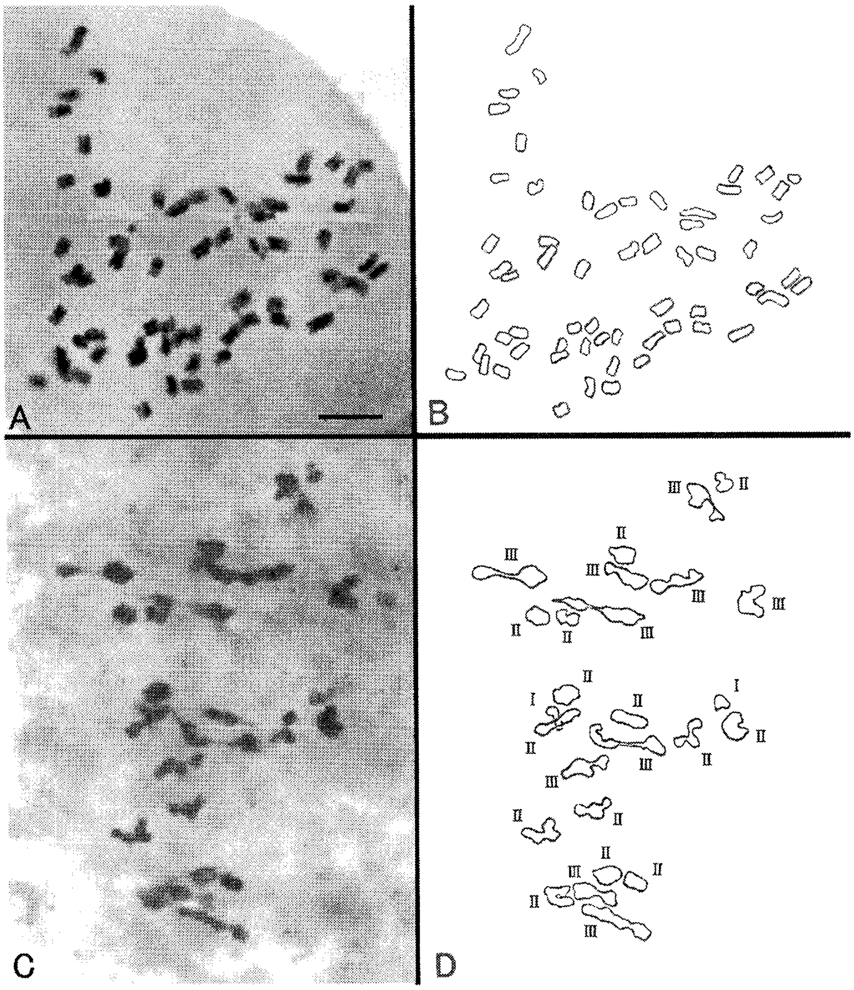

FIG. 3. Cytogenetic characteristics of the non-fruit-individuals of *Cayratia japonica*. A: Somatic chromosomes, $2n$=60, B: Explanatory drawings of Fig. 3A. C: $10_{III}+14_{II}+2_{I}$ at metaphase I, D: Explanatory drawings of Fig. 3C. Bar in A, 4 μm.

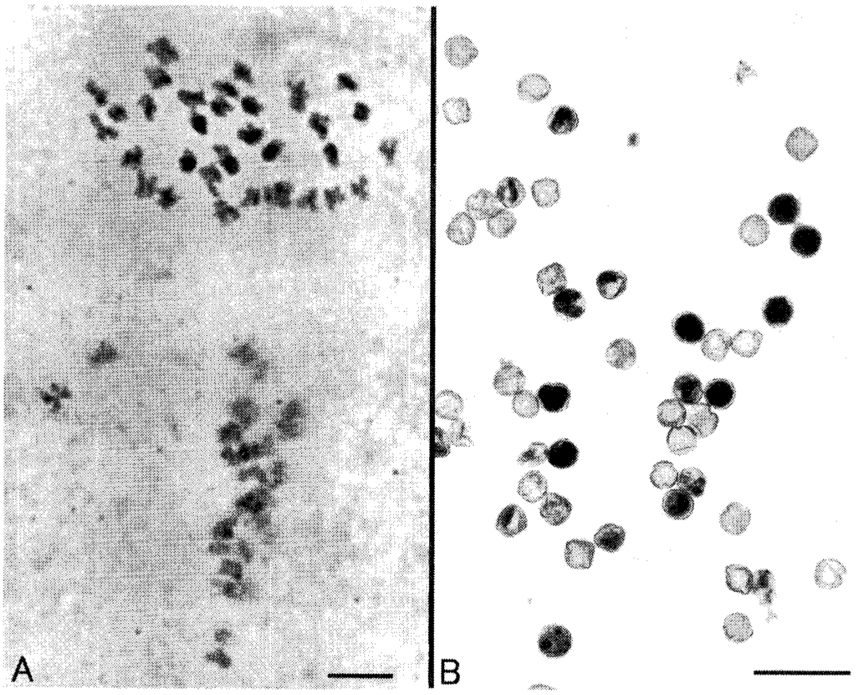

FIG. 4. Cytogenetic characteristics of the non-fruit-individuals of *Cayratia japonica*. A: 33 chromosomes in one of the nuclei at metaphase II, B: pollen of an individual with $2n$=60. Bar in A, 4 μm, in B, 100 μm.

concerned with pollen development were distributed on different chromosomes. If the gene combination was differentiated between both species, tetrads of the F_1 hybrid would contain a recombined gene combination. Such recombined genes acted incompatibly in pollen development, thus reducing the pollen fertility. In fact, Procissi et al. (2001) showed examples of multiple genes concerned with pollen development in *Arabidopsis thaliana*, and Fishman & Willis (2001) reported on the effect of recombined genes on the infertility of the next generation in *Mimulus*.

It is generally accepted that one of the very important steps in speciation is the establishment of an isolation mechanism between newly derived populations (for example; Stebbins 1950, Grant 1981). We have various examples showing reproductive isolation by mutation of the chromosomal structure, which results in abnormal chromosome segregation at meiosis and in presence of pollen sterility (for example; Okada 1981), or by genetic variation (Grant 1956). If the various pollen fertilities observed in diploid *Cayratia japonica* arose from an incompatible combination of genes concerned with pollen development, as is the assumption in the case of the inter-specific hybrid between *Ranunculus sirelifolius* and *R. chinensis, C. japonica* must be composed of multiple genetic types that affect the reproductive isolation between types. It is necessary to analyze whether there are any genetic types within the species, and whether multiple genes concerned with pollen development in this species have differentiated within the species.

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Received Nobember 14, 2002; accepted March 20, 2003